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(71) Applicant *(for all designated States except US)*: OKLAHOMA MEDICAL RESEARCH FOUNDATION [US/US]; 825 NE 13th Street, Oklahoma City, OK 73104 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: BING, Guoying [CN/US]; 4624 North Harvey Parkway, Oklahoma City, OK 73118 (US).

(74) Agents: HANSEN, Eugenia, S. et al.; Sidley & Austin, Suite 3400, 717 N. Harwood, Dallas, TX 75201 (US).

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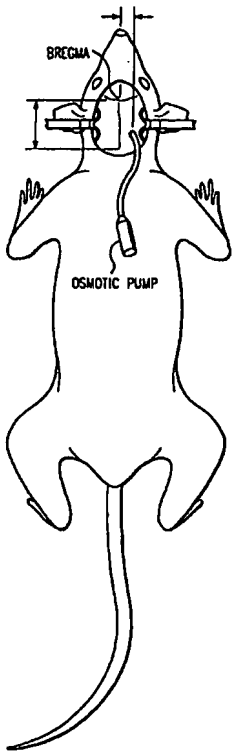
(54) Title: METHOD FOR PREVENTING AND TREATING THE DEGENERATION OF NEURONS

(57) Abstract

The present invention discloses a technique for inducing Parkinson-like symptoms in animals by injection of LPS into the substantive nigra. Moreover, we disclose a method for preventing and treating the degeneration of neurons in a region of the brain, in particular the degeneration of dopaminergic neurons in the substantia nigra, which is associated with Parkinson's disease.



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METHOD FOR PREVENTING AND TREATING THE DEGENERATION OF NEURONS

TECHNICAL FIELD OF THE INVENTION

This invention relates to a method for preventing and treating neurodegenerative diseases.

BACKGROUND OF THE INVENTION

The human central nervous system (CNS), which comprises the brain and spinal cord, is an intricate network of biological matter essential to human survival. The CNS coordinates the internal and external information associated with the human body by receiving, processing, and transmitting such information. The neuron (nerve cell) acts as the information processing and information transmitting component of the nervous system.

All neurons have a general organization that allows for electrical signals to be conducted along the neuron by taking advantage of the difference in electrical charge across the neuron's plasma membranes. Structurally, the neuron is comprised of a cell body, which contains the nucleus and cytoplasm, and the axon. The axon is an extensive outgrowth of the neuron capable of carrying an electrical signal away from the cell body toward a target cell or cells. Oligodendroglia cells, a type of neuronal support cell (glial cell), function to support axons of the CNS neurons by producing a myelin sheath. This sheath not only supports and insulates the axon but also assists with the travel of electrical current along the axon. Typically, for an electrical signal to be fully transmitted to the target cell or cells requires the employment of several neurons. The transfer of the electrical signal traveling along the axon of one neuron to another neuron occurs at a junction called the synapse. The axon transmits the electrical signal to the dendrites of another neuron by releasing a chemical messenger into the synapse.

A significant chemical messenger regarding the CNS is the catecholamine neurotransmitter, dopamine. It has been reported that dopamine comprises more than one-half of the catecholamine neurotransmitters residing in the CNS. Furthermore, dopamines' significance in the CNS is apparent after considering all of the activities to which dopamine is associated including: movement, sleep, mood, attention, and learning.

The synthesis of dopamine involves a two-step reaction starting from the essential amino acid L-tyrosine. The enzyme tyrosine hydroxylase catalyzes the hydrolysis of L-tyrosine to the dopamine precursor, L-Dopa. The hydroxylation of tyrosine is considered to be the rate-limiting step in the biosynthesis of dopamine. The enzyme L-aromatic amino acid decarboxylase, subsequently catalyzes the decarboxylation of L-Dopa to form dopamine. Formation of dopamine occurs within the terminal region of the dopaminergic neurons.

The formed dopamine is concentrated in storage vesicles at the terminal region of the dopaminergic neurons. The storage vesicles include membranes containing a high affinity , energy-dependent, carrier-mediated transport system. The transport system of the storage vesicles' membranes sequesters dopamine within the vesicles against a concentration gradient. Under physiological conditions, dopamine is released from dopaminergic neurons by a calcium-dependent process. Newly released dopamine into the synapse can bind to either a cell surface receptor for dopamine on the same dopaminergic neuron from which its was released or on another dopaminergic neuron. Dopamine released into the synaptic cleft is inactivated primarily by a high-affinity, carrier-mediated reuptake mechanism of the dopamine terminals. Thus previously released dopamine can again be sequestered against a concentration gradient and enter the storage vesicles for reuse.

As for most neurotransmitters, dopamine's "mode of operandum" is through specific dopamine receptors. That is the dopamine binds to a particular dopamine receptor to induce the appropriate response in another neuron or the target cell or cells. Two distinct types of dopamine receptors, D1 and D2, have been characterized based on their

pharmacological principles. Through molecular biology techniques, at least five genes encoding dopamine receptors have been isolated and identified. Therefore, five types of dopamine receptors have been identified to this date: the D1, D2, D3, D4, and D5 dopamine receptors. Each of these subtype dopamine receptors is categorized as either being D1-like or D2-like, depending on their nucleotide sequences and their pharmacological profile of the expressed proteins (receptors). The D1-like receptors include the D1 and the D5 receptors, whereas the D2-like receptors include the two isoforms of the D2 receptor, the D3 and the D4 receptors. The D1 and the D5 receptors activate the enzyme adenylyl cyclase, which stimulates the synthesis of the second messenger cyclic AMP and phosphatidyl inositol hydrolysis. The D2 receptors inhibit adenylyl cyclase activity, while additionally suppressing Ca^{2+} currents, and activating K^+ currents. The functional properties of the D3 and D4 receptors have not been identified at this time.

The majority of dopaminergic neurons reside mainly in the substantia nigra and striatum regions of the brain. In general, the cell bodies of the neurons are localized in the substantia nigra, while the terminal axons innervate the striatum region of the brain. For example, the pars compacta neurons of the substantia nigra provide dopaminergic input to the striatum, which comprises the caudate and the putamen. FIG. 1 illustrates the arrangement of these dopaminergic neurons.

Degradation of the dopaminergic neurons is a specific type of a neurodegenerative disorder resulting in the death of dopaminergic neurons. Upon neuronal death, astrocytes and microglial cells phagocytize the dead neurons. The measurement of microglial cell or astrocyte activity can serve as a method for quantifying the degeneration or death of the dopaminergic neurons as a result of a neurodegenerative disorder.

Neurodegenerative disorders, in general, are characterized by the progressive and irreversible loss of neurons within the brain. Generally speaking, the onset of neuro-degenerative disorders primarily appears at the later stages of an individuals life.

Parkinson's disease is a prototypical neurodegenerative disorder regarding the degeneration of dopaminergic neurons.

More specifically, Parkinson's disease is an extrapyramidal disorder comprising a variety of symptoms including tremor, rigidity, bradykinesia, and a recognizable disturbance in gait and posture. Parkinson's is chronic and generally commences in middle or late life. Further, Parkinson's is a progressive disability over time. The disease affects all ethnic groups and occurs equally in men and women. The frequency of the disease in the human population is one to two per one thousand in the general population and one per one-hundred among people older than sixty-five. The symptoms of Parkinson's disease are caused by the loss of dopaminergic neurons in the pigmented substantia nigra pars and the locus coeruleus in the midbrain. Dopaminergic neuron loss also occurs in the globus pallidus and putamen regions of the brain.

The mechanism causing the degradation of dopaminergic neurons in Parkinson's patients is speculative. A proposed mechanism is exposure to an unrecognized environmental toxin. The individuals exposure to such toxin could have occurred many years before the onset of any clinical disturbances because a cumulative dopaminergic neuron loss must approximate eighty percent of the original dopaminergic neuron population for Parkinson-like symptoms to be observable. Alternatively or additionally endogenous toxins may be a responsible mechanism.

FIG. 2 represents a model proposing the pathophysiological mechanism behind Parkinson's disease. It is thought that the loss of substantia nigra dopaminergic neurons causes a loss of excitation of the D1 striatal neurons in the direct pathway and loss of inhibition of the D2 striatal neurons in the indirect pathway. This combination of changes increases outflow of the neurotransmitter GABA (gamma-aminobutyric acid) from the substantia nigra pars reticulata and the globus pallidus interna. The increase in GABA, which innervates the thalamus, causes an increase in the inhibition of the thamocortical projections resulting in the loss of facilitation regarding cortically initiated movement. This

loss of cortically initiated movement results in the symptoms associated with Parkinson's disease.

Although the mechanism of any disease is best studied and understood by direct investigation of the human patient, such studies are often impractical, and therefore, experimental models are necessary. The use of animal models has become an integral part of the evaluation for the understanding of a disease and the development of a method and/or compound for treatment. Animal models can often be used as appropriate experimental models to assist in developing hypotheses concerning the fundamental process underlying the disease causing the degradation of dopaminergic neurons. Additionally, important information can be obtained from animal models including the biological properties and mechanism of action of the proposed therapeutic compound, synergistic activity with other compounds, and activities of analogs of the compound to be tested.

Common treatments for Parkinson's disease are for the most part purely symptomatic. The treatments include a variety of medicines based on either metabolic precursors for the neurotransmitter dopamine or dopamine agonists, which bind to and stimulate dopamine receptors causing a dopamine-type response. Table 1 presents a list of the various medicaments currently used for treating the symptoms associated with Parkinson's. A medicament, however, is needed for preventing the degeneration or further degeneration of the dopaminergic neurons.

Table 1

Drugs for Parkinson's Disease

(Goodman & Gilman, *The Pharmacological Basis of Therapeutics*, 9th Ed., p. 509, (McGraw-Hill, 1995))

AGENT	TYPICAL INITIAL DOSE	DAILY DOSE -- USEFUL RANGE
Carbidopa/levodopa	25 to 100 mg twice a day or three times a day	200 to 1200 mg levodopa

AGENT	TYPICAL INITIAL DOSE	DAILY DOSE -- USEFUL RANGE
Carbidopa/levodopa sustained release	50 to 200 mg twice a day	200 to 1200 mg levodopa
Pergolide	0.05 mg once a day	0.75 to 5.0 mg
Bromocriptine	1.25 mg twice a day	3.75 to 40 mg
Selegiline	5.0 mg twice a day	2.5 to 10 mg
Amantadine	100 mg twice a day	200 mg
Trihexyphenidyl HCl	1 mg twice a day	2 to 15 mg

It has now been found that the opiod antagonist, naloxone, may be administered to a patient in an effective amount to prevent and treat the degeneration of neurons in the brain. More specifically, naloxone prevents the degeneration of dopaminergic neurons in the striatum and substantia nigra regions of the brain. The method of using naloxone for this novel treatment comprises the administration of the known compound naloxone in an amount sufficient to prevent and treat the degeneration of neurons, in particular dopaminergic neurons.

SUMMARY OF THE INVENTION

The invention described herein provides a pharmaceutical composition commonly known as naloxone and a method for using naloxone to prevent and treat the degeneration of neurons by administering an effective amount of naloxone to a region of the brain affected by neuron degradation.

In another aspect of the invention, the method of administering naloxone can prevent the further degeneration of neurons associated with Parkinson's disease, in particular, when the naloxone is administered to the substantia nigra region of the brain.

In another aspect of the invention, a method is provided for creating lesions in neurons of an animal to generate an animal model for neuro-degenerative diseases. The lesions are created when an effective amount of lipopolysaccharide solution is administered to a region of the brain over a period of time.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a sagittal view of the brain illustrating the primary location for dopaminergic neurons.

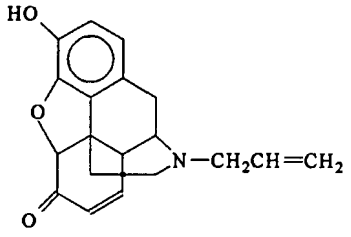
FIG. 2 is a schemata representing a model for the pathophysiological mechanism behind Parkinson's disease.

FIG. 3 is a sagittal view of the brain illustrating the injection of LPS into the region of the brain having a high concentration of dopaminergic neurons.

FIG. 4 is an aerial view of the insertion of the osmotic pump at a location superior to the substantia nigra and/or striatum region of the rat's brain.

DETAILED DESCRIPTION OF THE INVENTION

The invention described herein relates to the use of the compound naloxone and a method for using naloxone to prevent and treat the degeneration of neurons in the brain. Naloxone is an opiod antagonist for the opioid receptors $\delta,\kappa 1,\kappa 3,\mu$. The molecular structure for naloxone is:



(Goodman & Gilman, *The Pharmacological Basis of Therapeutics*, 9th Ed., p. 528 (McGraw-Hill, 1995)).

It has been found that the administration of a therapeutically effective amount of naloxone to an animal can prevent and treat the degeneration of neurons. A therapeutically effective amount of naloxone comprises the direct administration of about 220.0 to 450.0 micrograms of naloxone per kilogram of animal weight per day to a region of the brain affected by neuron degeneration.

The naloxone can be administered to any region of the brain affected by a neuro-degenerative disease. For example, Parkinson's is a neuro-degenerative disease that can be prevented and treated by the administration of naloxone to a Parkinson's patient. Thus, the continuous administration of naloxone can prevent further degeneration of the dopaminergic neurons and if administered timely, can prevent the onset of symptoms associated with Parkinson's disease.

Naloxone can be administered to the brain by subcutaneous implants, subcutaneous injection, or devices known to those skilled in the art for delivering a pharmaceutical to a designated region of the brain. With respect to Parkinson's disease, that region would be the substantia nigra.

A preferred method for delivering naloxone to the brain would be the use of a method that directly delivers the above mentioned amount of naloxone to the neurons affected by degeneration so to avoid premature metabolism of the naloxone. An example of such a method of delivery would be to insert a cannula into the region of the brain affected by neuron degeneration through a location of the head that has been locally anesthetized using a local anesthesia known to those skilled in the art of anesthesia. The cannula can then be connected to a device capable of delivering a solution of naloxone to the area of the brain affected by neuron degradation. Examples of such devices include

osmotic pumps, mechanical pumps, electrical pumps, and any other devices that can attach to the cannula and deliver a solution of naloxone to a target site in the brain.

Example 1 - General Animal Model

Animals to be tested were pathogen free and of a healthy body weight for their species. The animals were kept on a twelve (12) hour light/dark cycle and given food and water "ad libatum." Each animal was housed separately in their own cage.

The preferred anesthesia used was either the general anesthesia of 0.5 ml I.M. injection of a mixture of Ketamine (62.5 mg/Kg), xylene (3.25 mg/Kg), and acepromazine (0.62 mg/Kg) or the compound Numbital at 50 mg/Kg. Any anesthesia, however, capable of anesthetizing the animal without affecting the animal's survivability can be used. Additionally, all surgical procedures were performed under aseptic conditions.

After being properly anaesthetized, the animals were placed in a stereotaxic apparatus (David Kopf Instruments). The animals subsequently received a unilateral injection of anywhere between 2.0-10.0 μg of Lipopolysaccharides (LPS) (0111:B4; Sigma Chemical Company, St. Louis, MO) in 2.0 ml of 0.05 - 0.1 M phosphate buffer solution (PBS) at a location in the brain containing the neurons of interest, that is the neurons associated with a particular type of neurological disease. A preferred amount of LPS to be injected is 5.0 μg (approximately 2.2 μg of LPS/Kg of animal) with a preferred molarity of PBS at 0.1 M. The stereotaxic coordinates for injecting the LPS solution into the brain can be obtained from a brain atlas specific to the animal being studied. For example, an atlas of the rat's brain is mapped in the text *The Rat Brain*, Paxinos and Watson, Academic Press, 1997. The LPS is slowly injected into the particular region of the brain to allow proper diffusion. Five (5) minutes usually is an adequate time for the complete injection of LPS. The Hamilton Syringe (Catalog No. 80330) serves as a preferred apparatus for injecting the LPS but any injectable device of the appropriate size and capacity can suffice as long as the survivability of the animal is not jeopardized. After a period of time, usually between ten (10) and fourteen (14) days, the LPS induces a degeneration in the neurons located in the vicinity of the injected LPS.

A rotational model as illustrated in Bing et al. (Neuroscience 34:687-697, 1990), which is incorporated herein by reference, can be used to measure the physical effects of the unilateral LPS injection. If the animals already exhibit the symptoms of the neuro-degenerative disease and/or a NMR scan illustrates the degeneration of neurons in a particular region of the animal, the unilateral LPS injection can be skipped because the animal already exhibits Parkinson-like symptoms.

To enable the unequivocal identification of the degenerated neurons, the neurons of interest were first labeled by stereotaxic injections of a fluorescent tracer. A preferred time for labeling the neurons of interest is around seven (days) prior to the unilateral LPS injection. Although several fluorescent tracers are available, we used the tracer DiI (1,1'-Diactadecyl-3,3,3',3'-tetramethylindocarbocyanine; Molecular Probes, Inc., Junction City, Or). The tracer DiI is injected according to the techniques discussed in Hagg, T. and S. Varon, Proc. Nat'l Acad. Sci. 90:6315-19 (1993), which is incorporated herein by reference. Use of the DiI tracer/labels the neurons without affecting the survivability of the neurons *in vitro* and *in vivo* and remains detectable for several months *in vivo* without leaking into surrounding cells.

The animals exhibiting the symptoms of the particular disease and/or degeneration of the neurons of interest received implants from an appropriately sized cannula positioned at a region of the brain associated with the neuron degradation. The cannulas are connected to a device capable of delivering a solution of naloxone to the area of the brain affected by neuron degradation. Examples of such devices include osmotic pumps, mechanical pumps, electrical pumps, and any other devices that can attach to the cannula and deliver a solution of naloxone to a target site in the brain.

A preferred osmotic pump is the Alzet twenty (20) ounce pump (ALZA 2000). For the control group of animals, the osmotic pump contains only a solution of PBS. A preferred concentration of PBS is 0.1 M, however, any amount not affecting the results of the experiment can be used. These animals received a ten (10) day infusion of PBS. The osmotic pumps used for the non-control group animals contain a mixture of 0.1 M PBS and

a preferred concentration of 10^{-6} M naloxone. The naloxone concentration, however, can range between 10^{-6} - 10^{-7} M. These animals received a ten (10) day infusion of PBS and naloxone. Although a ten day infusion time is preferred, an infusion period between seven (7) - fourteen (14) days can be used.

Prior to use, the naloxone was stored in the dark at 4° C and brought to room temperature before being placed into the osmotic pump. In another practice of the invention, naloxone and PBS can be administered as a mixture with 60% ethanol. The naloxone concentration with this practice of the invention is between 0.05 - 0.1 mg/day, which is about 220.0-450.0 μg of naloxone/Kg/day. However, the preferred concentration is 0.1 mg/day, which is about 450.0 μg of naloxone/Kg/day. Although the preferred method for administering the naloxone is through a properly positioned metal cannula, the naloxone can also be administered by subcutaneous implants or subcutaneous injection.

After receiving either PBS or naloxone and PBS for the recommended time period, the animals were transcardially perfused with PBS followed by 4% paraformaldehyde post-fixation overnight. The brains were removed from the animals using techniques common to those skilled in the art then cryo-protected for three (3) days in 30% sucrose or a period of time sufficient to protect the brain tissue from deteriorating when frozen. After the brains were adequately cryo-protected, the brains were frozen for tissue sectioning. Symmetrical 30.0 µm-thick coronal sections of the affected brain region were cut on a freezing microtome. Somewhere around sixty sections were cut. Several of these sections were mounted and cover-slipped in fluoromount for analysis of DiI-labeled neurons. The neurons of interest were identified by using immunostaining techniques, which involved a product specific to the neurons. Adjacent sections of the brain were processed by using similar immunostaining techniques or by the microglial marker complement receptor, which employs the antibody CD 11b/c to bind with the CR3 complement receptor located on the microglia. The number of neurons positive for DiI on the specific immuno reactive product were counted in the region of the brain containing the neuron of interest in each section by the distribution of labeled neurons and a set of anatomical reference points. The diameters of twenty-five (25) neurons per animal were determined and used for correcting the total

number of neurons per section according to the formula N = n x T/(T+D), where N = total number, n = counted profiles, T = section thickness, D = longest cell diameter (Abercrombie, 1946). Microglial activation is assessed by assigning a semi-quantitative score on a scale of 0-5 with 1 being normal and 5 being the highest microglial activity. The neurons of interest were counted under the microscope and the sections of brains that received naloxone treatment were compared to those brains receiving only PBS. The results were evaluated by an analysis of variance (ANOVA) wherein the first factor considered was "group" and the second factor was "dosage".

Example 2 - Application of The Animal Model to Rats

Twelve (12) adult Sprague-Dawley specific pathogen free rats (Charles River) having an initial body weight of 200-225 g were selected. All of the rats were maintained on a 12 hour light/dark cycle with food and water provided *ad libatum*. The animals were housed individually in plastic cages covered with Envio-gard R bonnets. Each rat received an injection of the anesthetic Numbital (50 mg/kg) prior to surgery.

The anesthetized rats each received a unilateral injection of 5.0 μg of Lipopolysaccharides (LPS) (0111:B4; Sigma Chemical Company, St. Louis, MO) in 2.0 μl of phosphate buffered saline (PBS) 0.5 mm above the rostral third of the right substantia nigra pars compacta. The stereotaxic coordinates were the following: tooth bar set at -3.30 mm, lateral 1.70 mm from Bregma, caudal - 4.80 mm from Bregma, and ventral -7.60 mm from Bregma. The LPS injection extended for over a two (2) minute period using a Hamilton syringe. FIG. 3 illustrates the position of the Hamilton syringe in a rat brain. The LPS induced degeneration of the dopaminergic neurons in the vicinity of the LPS injection can be determined by performing immunocytochemistry regarding the enzyme tyrosine hydroxylase. A decrease in the level of tyrosine hydroxylase correlates with the degeneration of the dopaminergic neuron.

Immediately after completing the LPS injections, all the animals were implanted with the tip of a 0.30 mm in diameter and 7.0 mm long metal cannula (Alza 2002) immediately above the substantia nigra region of the brain for a 14-day infusion. The

stereotaxic coordinates are 1.7 mm lateral from Bregma, 4.10 mm caudal from Bregma and 7.00 mm ventral from Bregma. The cannulas are connected to an Alzet 2002 pump (12.0 µl/day; Alza 2002). Six (6) of the LPS injected animals received a 12.0 µl/day infusion of 0.1 M PBS containing 10^{-6} M naloxone (Sigma, St. Louis, MO). The other six (6) LPS injected animals receive a 12.0 µl/day infusion containing strictly 0.1 M PBS. FIG. 4 illustrates the location of the osmotic pump in reference to the rat. In another embodiment of the invention, naloxone and PBS were administered simultaneously using an Alzet pump filled with 60% ethanol or ethanol plus 0.1 mg/day naloxone.

To enable the unequivocal identification of the dopaminergic substantia nigra pars compacta neurons, the neurons were retrogradely labeled by stereotaxic injection of the fluorescent tracer DiI (1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine; Molecular Probes, Inc., Junction City, OR) one (1) week prior to naloxone treatment. The tracer was injected according to Hagg and Varon (which is incorporated by reference) into six tracts of each striatum two weeks before any other manipulation. (Hagg, T. and S. Varon, *Proc. Natl. Acad. Sci.* 90:6315-19 (1993)). This method labels all nigrostriatal neurons, which are all immunoreactive for the enzyme tyrosine hydroxylase.

After ten (10) days of either receiving PBS or naloxone and PBS, the animals were transcardially perfused with PBS followed by 4% paraformaldehyde postfixation overnight. The brains of the rats were surgically removed and then cryoprotected for three (3) days prior to frozen tissue sectioning. Symmetrical 30.0 µm-thick coronal sections were cut on a freezing microtome and every sixth section through the nigral complex was mounted and coverslipped in fluoromount for analysis of DiI-labeled neurons. The dopaminergic nigrostriatal neurons in the substantia nigra pars compacta were identified by immunostaining of adjacent sections for tyrosine hydroxylase (TH) as demonstrated by Bing, et al., *Neuroscience*, 34:687-697 (1990), which is herein incorporated by reference. Adjacent sections were processed for TH immunocytochemistry (1:40,000 MAb318 antibody; Chemicon Inc. Temecula, CA) or for the microglial marker complement receptor 3 (1:20,000 OX-42 antibody). The number of neurons positive for DiI or TH was counted in substantia nigra pars compacta regions defined in each section by the distribution of

labeled neurons and a set of anatomical reference points. The diameters of 25 neurons per animal were determined and used for correcting the total number of neurons per section according to the Abercrombie formula $N = n \times T / (T + D)$, where N = total number, n = counted profiles, T = section thickness, D = longest cell diameter. The extent of microglial activation was assessed by assigning a semi-quantitative score on a scale of 0-5 with 1 being normal and 5 being most extensive. The number of nigral neurons was counted under the microscope to compare the effects of naloxone (both local and systemic administration). The results were evaluated by ANOVA and demonstrated that naloxone protects the substantia nigra dopaminergic neurons from LPS-induced neurotoxicity.

I CLAIM:

1. A method for preventing and treating the degeneration of neurons comprising administering to an animal an effective amount of naloxone to a region of the brain affected by neuron degeneration.

2. A method according to claim 1 wherein said effective amount of naloxone is in the range of 10^{-6} - 10^{-7} M.

3. A method according to claim 1 wherein said effective amount of naloxone is of a ratio of about 220.0-450.0 micrograms of naloxone per kilogram of animal weight per day.

4. A method according to claim 1 wherein said effective amount of naloxone is continuously administered to a region of the brain affected by neuron degeneration.

5. A method according to claim 1 wherein the prevention of neuron degeneration is for dopaminergic neurons.

6. A method according to claim 1 in which the administration of an effective amount of naloxone for patients affected by Parkinson's disease prevents the further degeneration of neurons associated with Parkinson's disease.

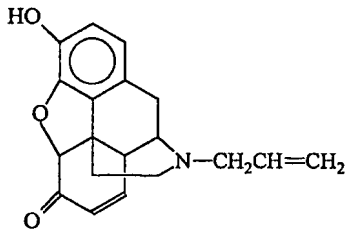
7. The method according to claim 6 wherein said effective amount of naloxone is administered in the substantia nigra region of the brain of a patient affected by Parkinson's disease.

8. The method according to claim 4 in which said effective amount of naloxone is continuously administered subcutaneously.

9. A method according to claim 4 wherein said effective amount of naloxone is continuously administered by subcutaneous implants located in the region of the brain associated with neuron degeneration.

10. A pharmaceutical composition for preventing and treating the degeneration of neurons comprising the administration of a therapeutically effective amount of the compound naloxone.

11. A pharmaceutical composition according to claim 10 in which said pharmaceutical composition is of the formula:



12. A pharmaceutical composition according to claim 10 wherein said therapeutically effective amount of naloxone is of a ratio of about 220.0-450.0 micrograms of naloxone per kilogram of animal weight per day.

13. A pharmaceutical composition according to claim 10 wherein said therapeutically effective amount of naloxone is delivered in a solution of phosphate buffer having a concentration at about 0.1M.

14. A pharmaceutical composition according to claim 12 wherein said therapeutically effective amount of naloxone is delivered in a solution of phosphate buffer at a concentration of about 0.1m, said solution further comprising 60% ethanol.

15. A pharmaceutical composition according to claim 10 wherein said effective amount of naloxone is continuously administered to prevent further degeneration of neurons.

16. A method for creating lesions in neurons of an animal comprising the administration to a region of the brain to be investigated an effective amount of a lipopolysaccharides solution.

17. A method according to claim 16 wherein said effective amount of lipopolysaccharides solution comprises 2.0-10.0 micrograms of lipopolysaccharides.

18. A method according to claim 16 wherein said effective amount of lipopolysaccharides solution injected is of a ratio of about 2.2 micrograms of lipopolysaccharides per kilogram of animal weight.

19. A method according to claim 16 further comprising the testing for lesions in animals administered said effective amount of said lipopolysaccharides solution using a rotational model.

20. A method for preventing and treating the degeneration of neurons comprising continuously administering to an animal 220.0-450.0 micrograms of naloxone per kilogram of animal weight per day to a region of the brain affected by neuron degeneration, said naloxone is delivered in a solution of phosphate buffer having a concentration of about 0.1M.

21. A pharmaceutical composition for preventing and treating the degeneration of neurons comprising the continuous administration of about 220.0-450.0 micrograms of naloxone per kilogram of animal weight per day to a region of the brain affected by neuron degeneration, said naloxone is delivered in a solution of phosphate buffer having a concentration at about 0.1M.

22. A method for creating lesions in neurons on an animal comprising the administration to a region of the brain to be investigated a lipopolysaccharides solution comprising 2.0-10.0 micrograms of lipopolysaccharides wherein said administered lipopolysaccharide solution is of a ratio of about 2.2 micrograms of lipopolysaccharides per kilogram of animal weight.

FIG. 1

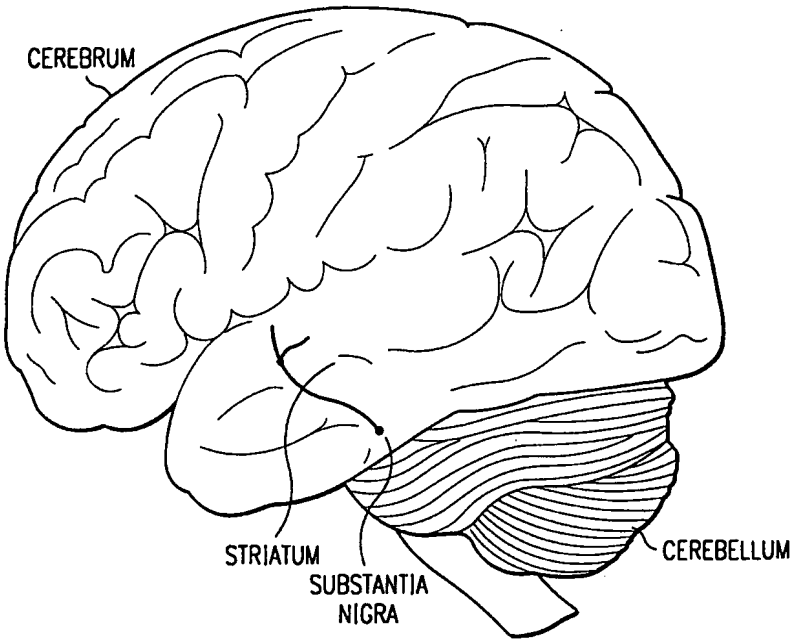


FIG. 2

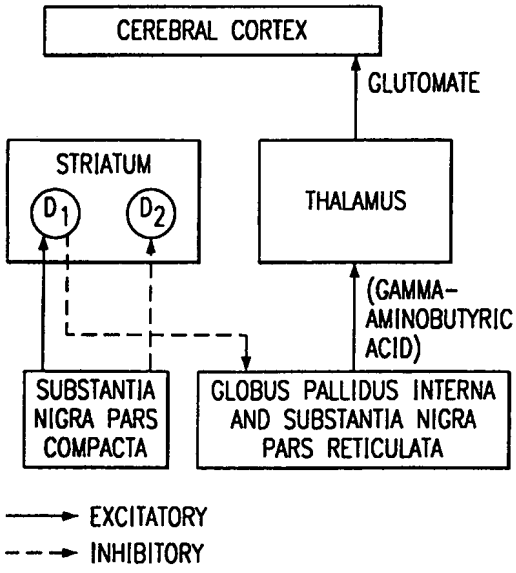
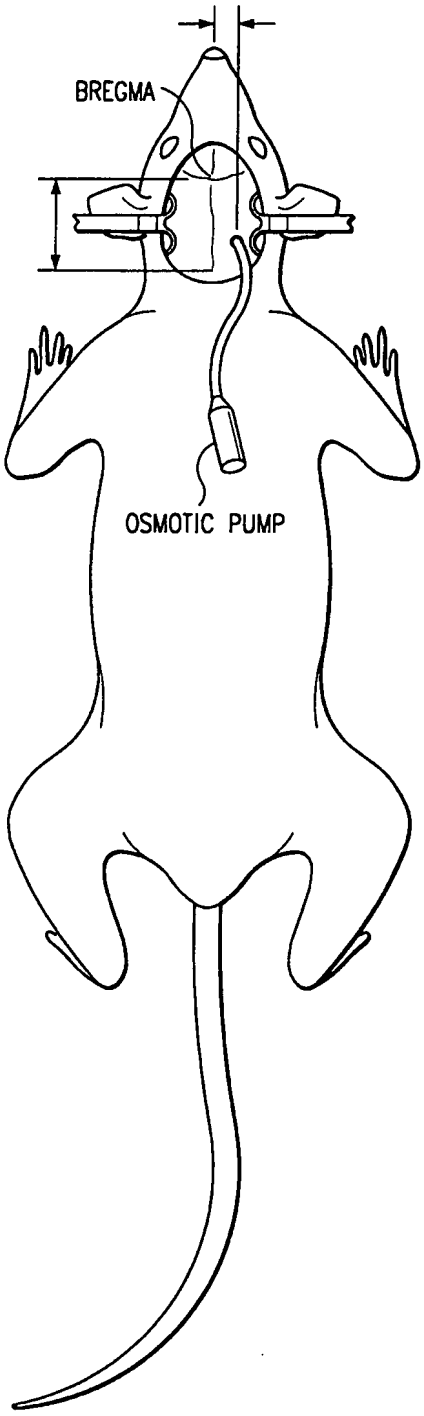

CEREBRAL CORTEX
GLUTOMATE
STRIATUM
D1
D2
THALAMUS
(GAMMA-AMINOBUTYRIC ACID)
SUBSTANTIA NIGRA PARS COMPACTA
GLOBUS PALLIDUS INTERNA AND SUBSTANTIA NIGRA PARS RETICULATA
EXCITATORY
INHIBITORY

FIG. 4


BREGMA
OSMOTIC PUMP

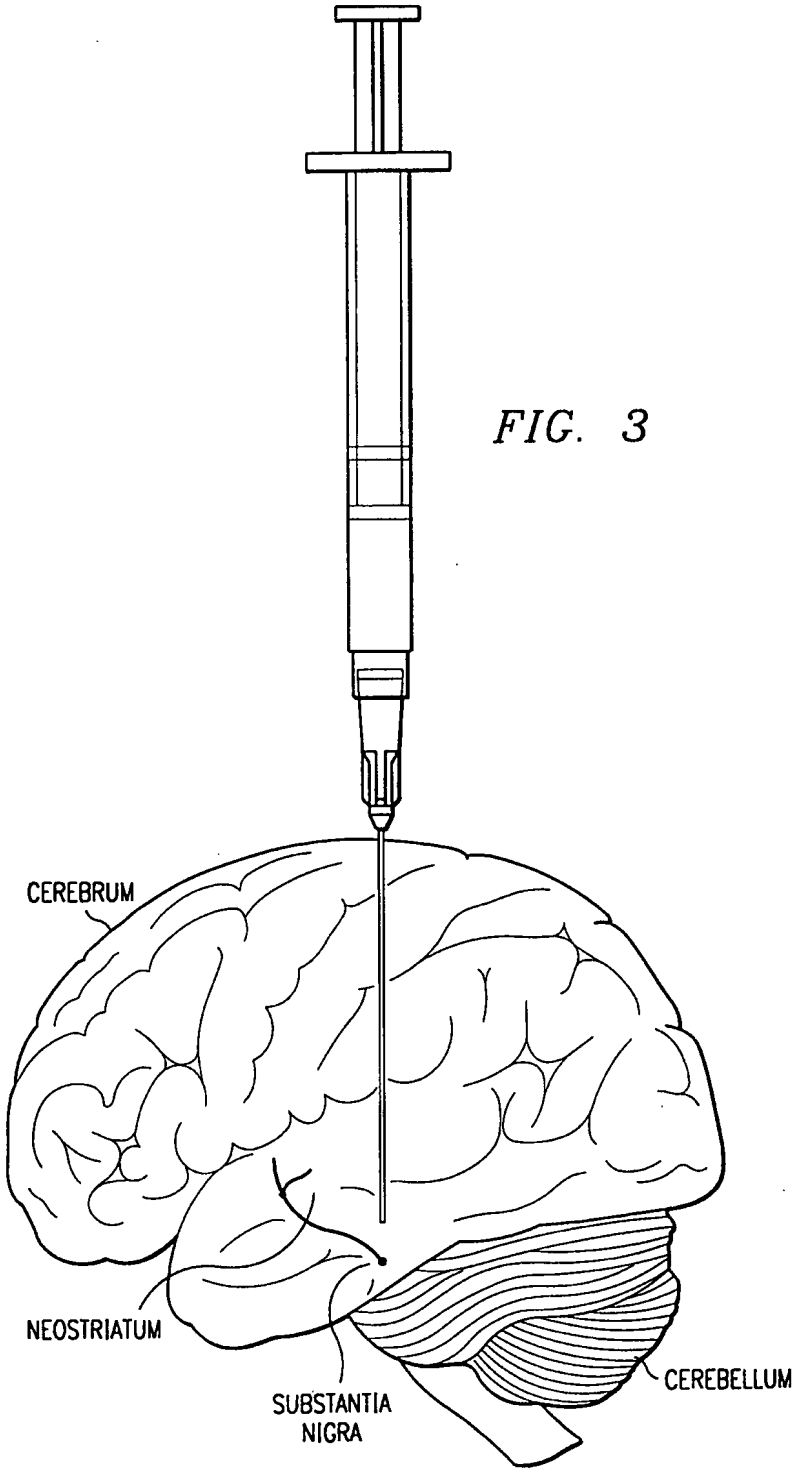


FIG. 3